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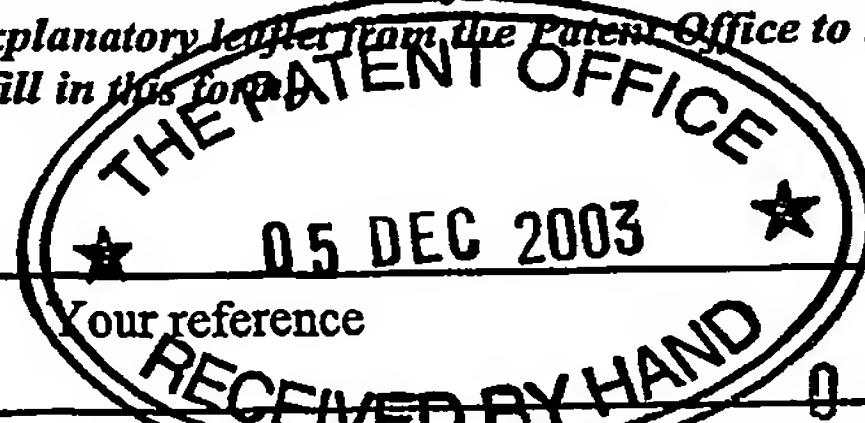
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2. Patent application number 0328321.5
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Cambridge Biotechnology Limited
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UNITED KINGDOM

Patents ADP number (if you know it) 08208761 002If the applicant is a corporate body, give the
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UNITED KINGDOM

4. Title of the invention

Synthesis of 2-substituted Adenosines

5. Full name, address and postcode in the United
Kingdom to which all correspondence relating
to this form and translation should be sent

Reddie & Grose
16 Theobalds Road
LONDON
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Synthesis of 2-Substituted Adenosines

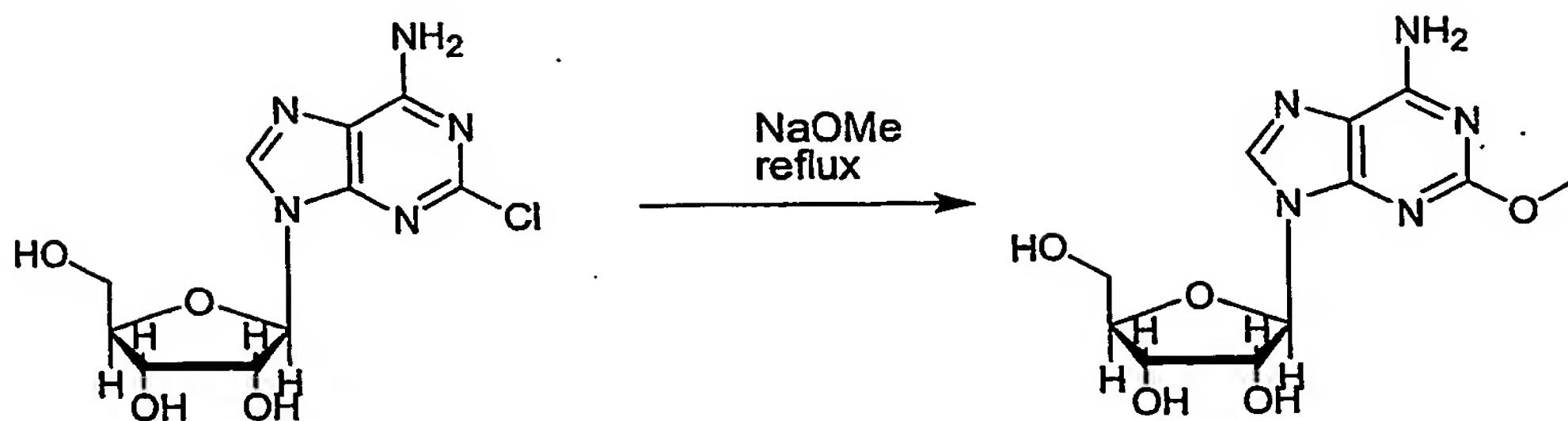
This invention relates to synthesis of 2-substituted adenosines, such as spongosine (2-methoxyadenosine) and to synthesis of intermediates for use in the synthesis of such compounds.

The natural product spongosine was first isolated from a sponge, *Cryptotethia crypta*, collected off the Florida coast in 1945 (Bergmann and Feeney, J.Org. Chem. 1951, 16, 981; Ibid 1956, 21, 226). Spongosine was considered an unusual nucleoside in that it was not only the first methoxypurine to be found in nature but also one of the first O-methyl compounds to be isolated from animal tissues.

Several syntheses of spongosine have been previously reported. One of the first of these to be published was by Bergmann and Stempien (J. Org. Chem. 1957, 22, 1575) in which spongosine was formed via coupling of chloromeric 2-methoxyadenosine to 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride. This simple coupling reaction provided a crude yield of spongosine of 31% which was then recrystallised from hot water to provide spongosine which exhibited a melting point of 191-191.5°C and an optical rotation of -43.5° (NaOH).

A variation on this theme was employed by Ojha *et al.* (Nucleosides and Nucleotides, 1995, 14, 1889) who initially coupled 2-ethylthioadenine with a suitably protected ribose. Subsequent adjustments of the protecting groups and oxidation gave a substrate which was reacted with sodium methoxide to yield spongosine in a yield of 87% for the final step. The purity of the target spongosine after column chromatography clean up, was proved by both elemental analysis and melting point (189-190°C).

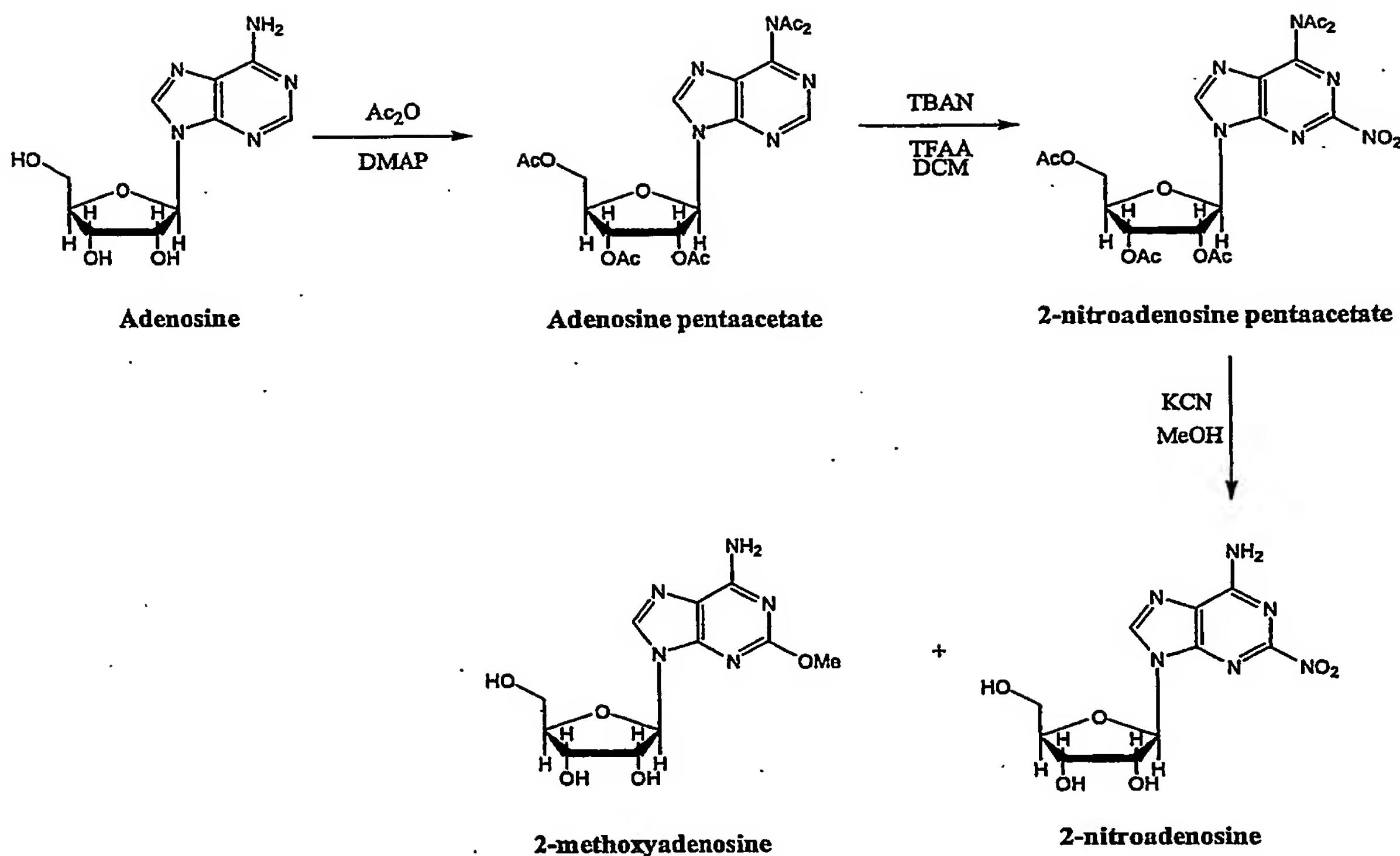
One of the most common methods of preparation of spongosine is via displacement of a 2-substituted chlorine atom by methoxide:



This methodology has been successfully applied by a number of groups to provide spongosine in varying yields and purity: Schaeffer *et al.*; J. Am. Chem. Soc. 1958, 80, 3738 (35% yield, mpt. 190-192°C); Bartlett *et al.*; J. Med. Chem. 1981, 24, 947 (yield and purity not quoted), Sato *et al.*; Synth. Proceed. Nucleic Acid Chem. 1968, 1, 264. However, this method suffers from the disadvantage that the 2-chloroadenosine starting material is difficult to synthesise and expensive.

Spongosine was reported by Cook *et al.* (J. Org. Chem. 1980, 45, 4020) as a by-product in the methylation reaction of isoguanosine by methyl iodide. Both the desired 1-methylisoguanosine and the spongosine were obtained in poor crude yields (19 and 30% respectively). The crude spongosine fragment was first purified by column chromatography on silica gel (eluent: chloroform/methanol) and then recrystallised from water to provide a sample which melted between 189-192°C (7% yield pure).

Paymaneh *et al* (Tetrahedron Letters 41 (2000) 1291-1295) and Wanner *et al.* (Bioorganic & Medicinal Chemistry Letters 10 (2000) 2141-2144) describe formation of spongosine as a significant by-product in the synthesis of 2-nitroadenosine by treatment of 2-nitroadenosine pentaacetate with potassium cyanide in methanol. The 2-nitroadenosine was obtained in only 10% yield, and spongosine in 47% yield (Paymaneh *et al.*). The 2-nitroadenosine pentaacetate was produced by nitration of pentaacetylated adenosine with tetrabutylammonium nitrate/trifluoroacetic anhydride (TBAN/TFAA), and (in Wanner *et al.*) the pentaacetylated adenosine was formed by treatment of adenosine with acetic anhydride and DMAP:



Synthesis of spongiosine (2-methoxyadenosine) according to Wanner *et al.*

A disadvantage of this method is that the spongiosine is not produced in high yield or purity. A further disadvantage of the method is that it involves use of the toxic reagent potassium cyanide. It is desired, therefore, to provide alternative methods of synthesis of spongiosine, and to improve the yield and purity of the spongiosine produced.

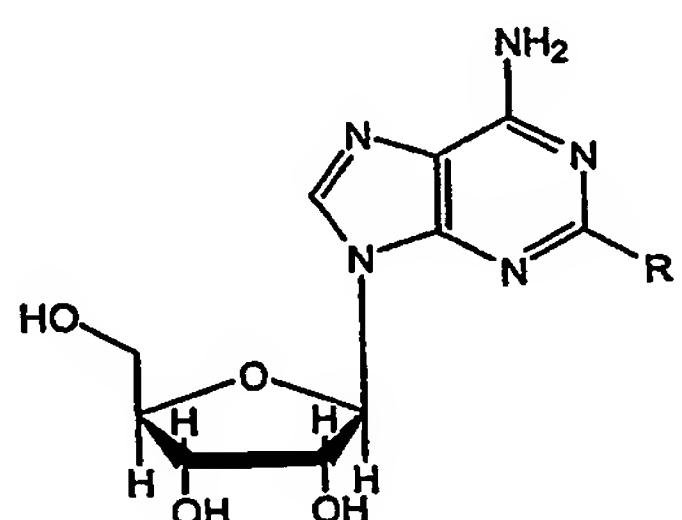
We have appreciated that the yield and purity of spongiosine produced by the method of Paymaneh *et al.*, and Wanner *et al.* is limited by a number of factors:

- i) The 2-nitroadenosine pentaacetate is contaminated with TBAN which adversely affects the purity and yield of the spongiosine product. This is particularly problematic because TBAN is amphiphilic, and so could not be removed by aqueous work-up. In addition, because of the partial solubility of 2-nitroadenosine pentaacetate in the aqueous layer, some of this may have been lost.
- ii) The adenosine pentaacetate intermediate is produced only in low yield and purity. We found that the tetra-acetylated precursor is present as a major by-product.
- iii) The fifth acetate group of the penta-acetyl compounds is labile, and this results in decomposition of these compounds to tetra-acetyl compounds. For example, we purified adenosine pentaacetate by column chromatography, but there was evidence to

suggest that the compound decomposed during this process. Attempts to recrystallise this compound were not successful and it was amorphous rather than crystalline in nature.

We have found, surprisingly, that the purity and yield of spongosine and other 2-substituted adenosines may be greatly improved by use of benzoyl protecting groups.

According to the invention there is provided a method of synthesis of a 2-substituted adenosine of formula I, which comprises converting 2-nitro pentabenzoyl adenosine to the 2-substituted adenosine:



I

wherein R = C₁₋₆ alkoxy (straight or branched), a phenoxy group (unsubstituted, or mono-, or di-substituted by halo, amino, CF₃-, cyano, nitro, C₁₋₆ alkyl, or C₁₋₆ alkoxy) or a benzoyl group (unsubstituted, or mono-, or di-substituted by halo, amino, CF₃-, cyano, nitro, C₁₋₆ alkyl, or C₁₋₆ alkoxy).

Preferably R = methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, phenoxy, or benzoyl.

We have found that 2-nitro-pentabenzoyl adenosine has increased organic solubility, stability and crystallinity compared to 2-nitroadenosine pentaacetate. The 2-nitro-pentabenzoyl adenosine is, therefore, easier to handle than 2-nitroadenosine pentaacetate, and can be made in higher yield and purity than this compound. The yield and purity of the spongosine produced is thereby also improved. Other 2-substituted adenosines can also be produced in high yield and purity using 2-nitro-pentabenzoyl adenosine as intermediate.

Preferably the 2-nitro-pentabenzoyl adenosine is converted to the 2-substituted adenosine by deprotecting the 2-nitro-pentabenzoyl adenosine and reaction with a suitable anion (for example C₁₋₆ alkoxide anion, or a phenoxide anion). To synthesise spongosine this may be achieved by reaction with potassium cyanide and methanol as detailed in Paymaneh *et al.*, and Wanner *et al.* However, it is preferred that less toxic sources of the methoxide anion are used. Preferred sources are MeOH/NaOMe, MeOH/n-BuLi, MeOH/NaOH, or MeOH/NaH.

A preferred method of methoxylating 2-nitro-pentabenzoyl adenosine is described in Example 4 below.

Other 2-substituted adenosines of formula I may be made by treatment of 2-nitro-pentabenzoyl adenosine with sodium hydroxide and an appropriate alcohol (for example C₁₋₆ alcohol, or phenol).

Preferably methods of the invention further comprise converting pentabenzoyl adenosine to 2-nitro-pentabenzoyl adenosine.

According to a further aspect of the invention, there is provided a method of synthesising 2-nitro-pentabenzoyl adenosine or a 2-substituted adenosine of formula I, which comprises converting pentabenzoyl adenosine to 2-nitro-pentabenzoyl adenosine.

Conversion of pentabenzoyl adenosine to 2-nitro-pentabenzoyl adenosine may be achieved by nitrating pentabenzoyl adenosine with a suitable nitrating reagent, such as tetrabutylammonium nitrate (TBAN) or tetramethylammonium nitrate (TMAN). Preferably nitration is carried out using TBAN or TMAN with trifluoroacetic anhydride (TBAN/TFAA, or TMAN/TFAA). Preferably the TBAN/TFAA or TMAN/TFAA is in dichloromethane (DCM).

2-nitro-pentabenzoyl adenosine has increased organic solubility and crystallinity compared to 2-nitroadenosine pentaacetate. A particular advantage of these properties is that, in contrast to 2-nitroadenosine pentaacetate, much of the TBAN or TMAN can

be removed from the 2-nitro-pentabenzoyl adenosine by aqueous work-up, preferably followed by recrystallisation. Preferably 3-5 washes are carried out in the aqueous work-up, and preferably 2 or 3 recrystallisations are carried out.

For example, aqueous work-up of the 2-nitro-pentabenzoyl adenosine produced may be carried out by dissolving the compound in an organic solvent (such as ethyl acetate or DCM), and washing the resulting solution with water. In general, a minimum of three washes has been found to be required to remove a large proportion of the TBAN or TMAN. However, five washes are generally carried out to ensure as much TBAN or TMAN as possible is removed.

Recrystallisation may be carried out by removing the organic solvent after the solution has been washed with water, dissolving the 2-nitro-pentabenzoyl adenosine in EtOAc/ethanol, or dichloromethane/ethanol, and crystallising the 2-nitro-pentabenzoyl adenosine from this solution.

We have found that the crude product of the nitration reaction with TBAN/TFAA could not be recrystallised because of the large amount of TBAN present. However, after aqueous work-up the compound could be readily recrystallised from a mixture of EtOAc and ethanol. Impurities other than TBAN were also present in the mixture after the work-up process and these could be removed by recrystallisation. A minimum of one recrystallisation may be sufficient but sometimes two or three recrystallisations may be required for satisfactory removal of these impurities.

The increased organic solubility of the penta-benzoyl compounds compared with the penta-acetyl compounds ensures that only an insignificant amount of compound is lost by aqueous work-up and recrystallisation.

Preferred methods of nitrating pentabenzoyl adenosine are described in Examples 3 and 4 below.

Preferably methods of the invention further comprise converting adenosine to pentabenzoyl adenosine.

According to the invention there is further provided a method of synthesising pentabenzoyl adenosine, 2-nitro-pentabenzoyl adenosine, or a 2-substituted adenosine of formula I, which comprises converting adenosine to pentabenzoyl adenosine.

Conversion of adenosine to pentabenzoyl adenosine may be achieved by benzoylating adenosine with a suitable benzoylating reagent, such as benzoyl chloride. dimethylformamide (DMF) may be used as solvent, but preferably the adenosine is dissolved/suspended in pyridine as this gives cleaner results.

A preferred method of benzoylating adenosine is described in Example 1 below.

An advantage of use of pentabenzoyl adenosine is that it can be more readily purified than adenosine pentaacetate. For example, pentabenzoyl adenosine was purified by aqueous work-up followed by recrystallisation. This was preferable to purification of adenosine pentaacetate which involved column chromatography during which some decomposition and loss of product occurred.

There is also provided according to the invention use of pentabenzoyl adenosine in the synthesis of 2-nitro pentabenzoyl adenosine, or a 2-substituted adenosine of formula I.

There is further provided according to the invention use of a benzoylating reagent in the synthesis of a 2-substituted adenosine of formula I.

There is also provided according to the invention a 2-substituted adenosine, 2-nitro-pentabenzoyl adenosine, or pentabenzoyl adenosine synthesised by a method of the invention.

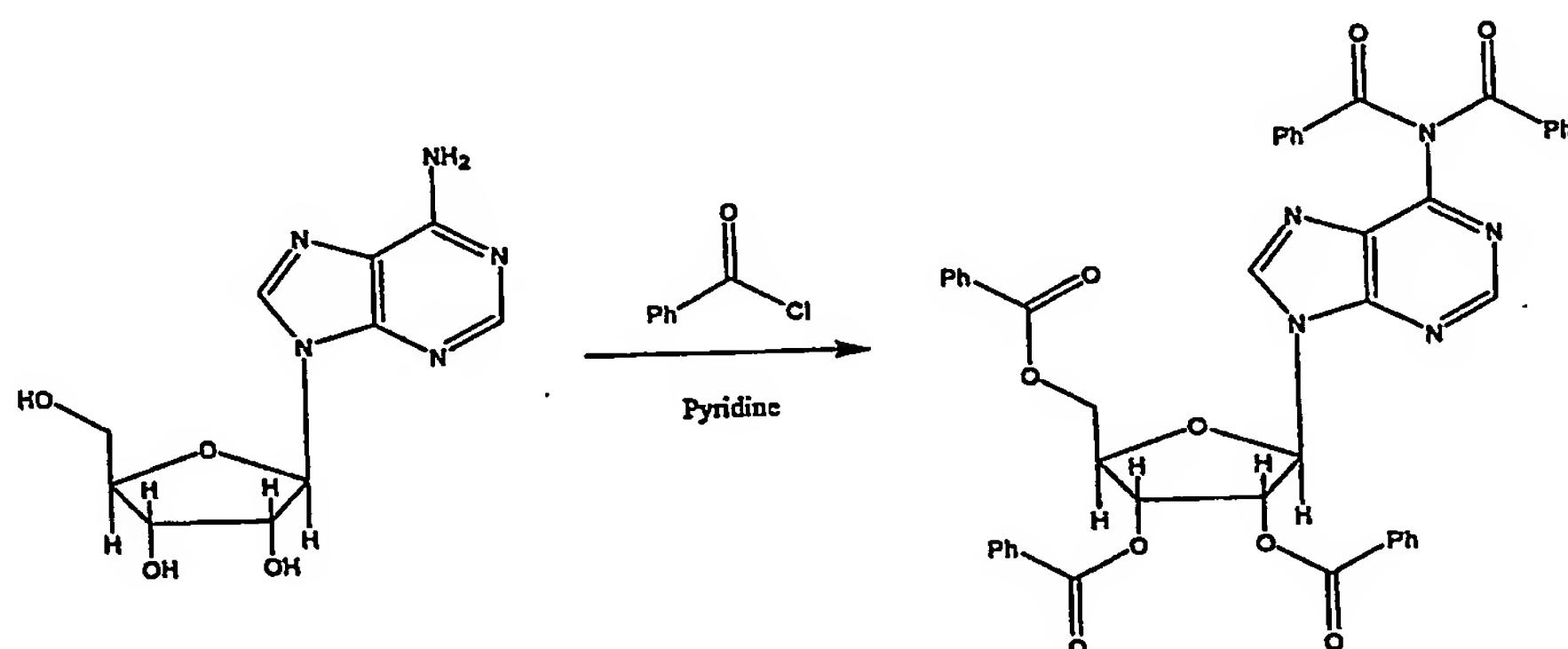
Methods of the invention allow synthesis of products more easily, and with greater yield and purity than the known method of Paymaneh *et al.* and Wanner *et al.* which uses acetyl protecting groups. We have appreciated that this is due to the increased organic solubility, stability and crystallinity of the compounds used in the invention.

Using acetyl as protecting group we were able to synthesise spongiosine on a 50mg scale. However, we have been able to synthesise spongiosine on a 100mg scale using benzoyl as protecting group according to methods of the invention.

Embodiments of the invention are now described by way of example only.

Example 1

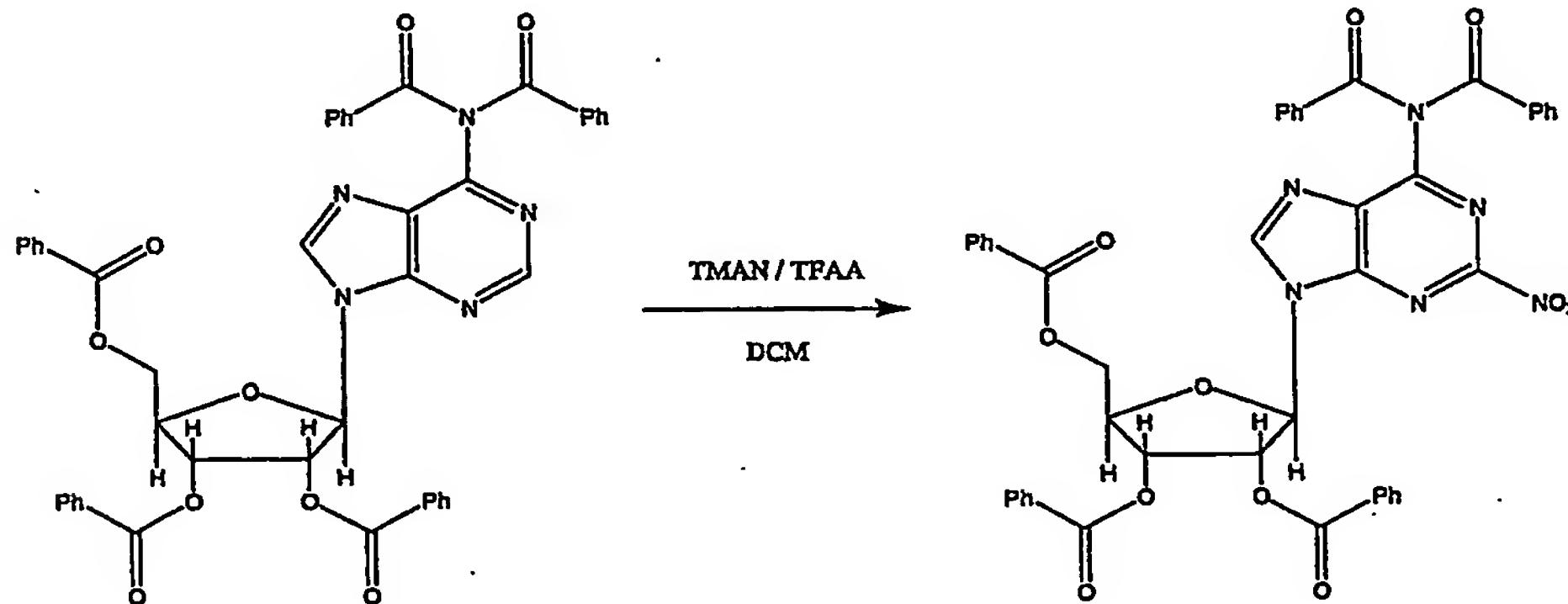
Preparation of Pentabenzoyl Adenosine:



To a suspension/solution of adenosine (2.00g, 7.47 mmol) in pyridine (20 cm³) add benzoyl chloride (7.35g, 6.07 cm³, 52.29 mmol). Heat at 65 °C for 4h, pour reaction mixture onto ethanol (20 cm³). Solvent removed *in vacuo*. Residue partitioned between DCM (300 cm³), washed with water (100 cm³), aqueous layer washed with DCM (3 × 50 cm³), organic layers combined and washed with water (2 × 100 cm³), brine (100 cm³), dried (MgSO₄). Solvent removed *in vacuo*, residue purified by recrystallisation from acetone/EtOH to give the desire product (5.660 g, 96.2 %) as a colourless solid. LCMS: 788 (M + H).

Example 2

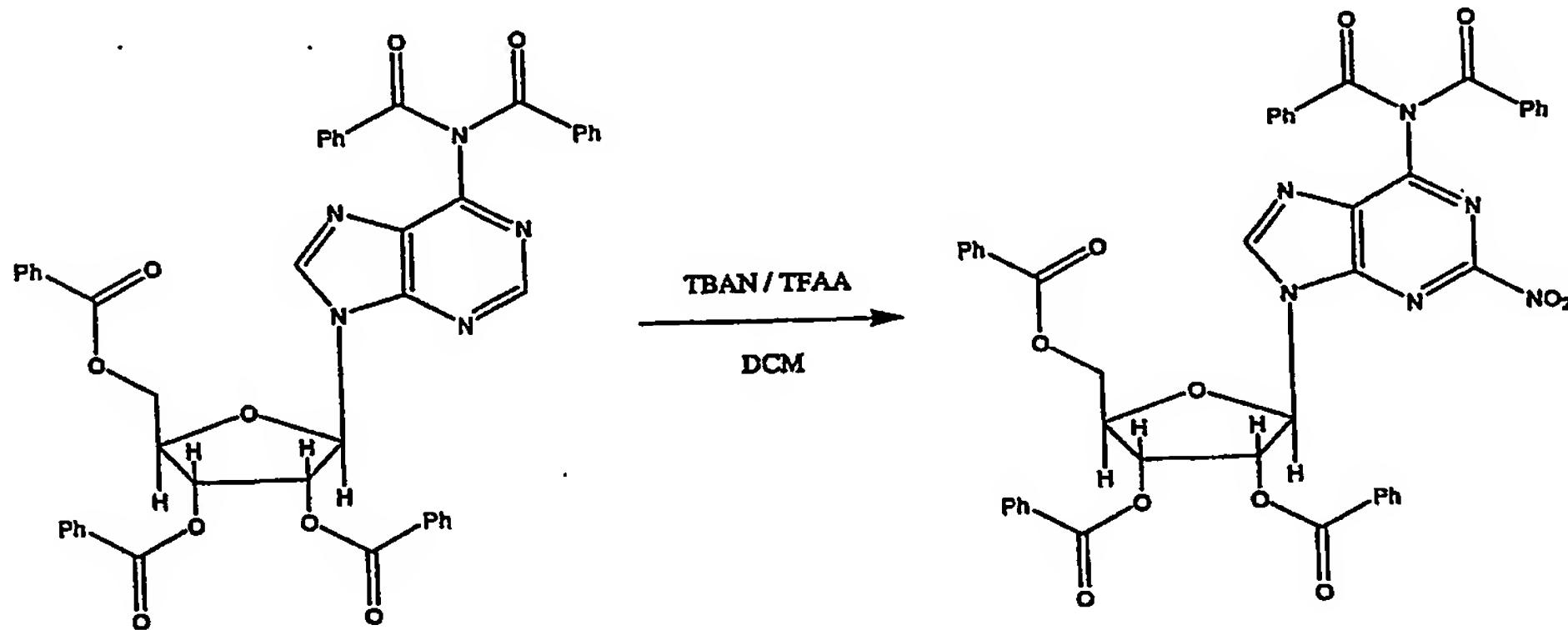
Preparation of 2-Nitro-Pentabenzoyl Adenosine using TMAN/TFAA as nitrating reagent:



To a suspension of tetramethylammonium nitrate (1.37 g, 11.4 mmol) in DCM (40 cm³) charge trifluoroacetic anhydride (2.40 g, 1.62 cm³, 11.4 mmol). Stir at room temperature for 1.5h, cool to 0 °C and add a solution of pentabenzoyl adenosine (6.00 g, 7.62 mmol) in DCM (50 cm³). Allow to warm to room temperature over 14h, solvent removed *in vacuo* [Temperature of rotary evaporator water bath is kept at 30 °C or below]. Residue dissolved in EtOAc (200 cm³), washed with water (3 × 150 cm³), brine (50 cm³), dried (MgSO₄). Solvent removed *in vacuo*, residue purified by recrystallisation from DCM/EtOH (twice) to give the desire product (5.59 g, 88.2 %) as an off white solid. ¹H NMR (400MHz, CDCl₃): 4.79 (1H, dd, J = 11.5, 4.2 Hz), 4.92 (2H, m), 6.08 (1H, t, J = 5.6 Hz), 6.16 (1H, dd, J = 5.8, 4.4 Hz), 6.57 (1H, d, J = 5.4 Hz), 7.39 (10H, m), 7.55 (5H, m), 7.85 (4H, m), 7.92 (2H, m), 8.04 (4H, m) and 8.44 (1H, s). LCMS: 833 (M + H) and 855 (M + Na).

Example 3

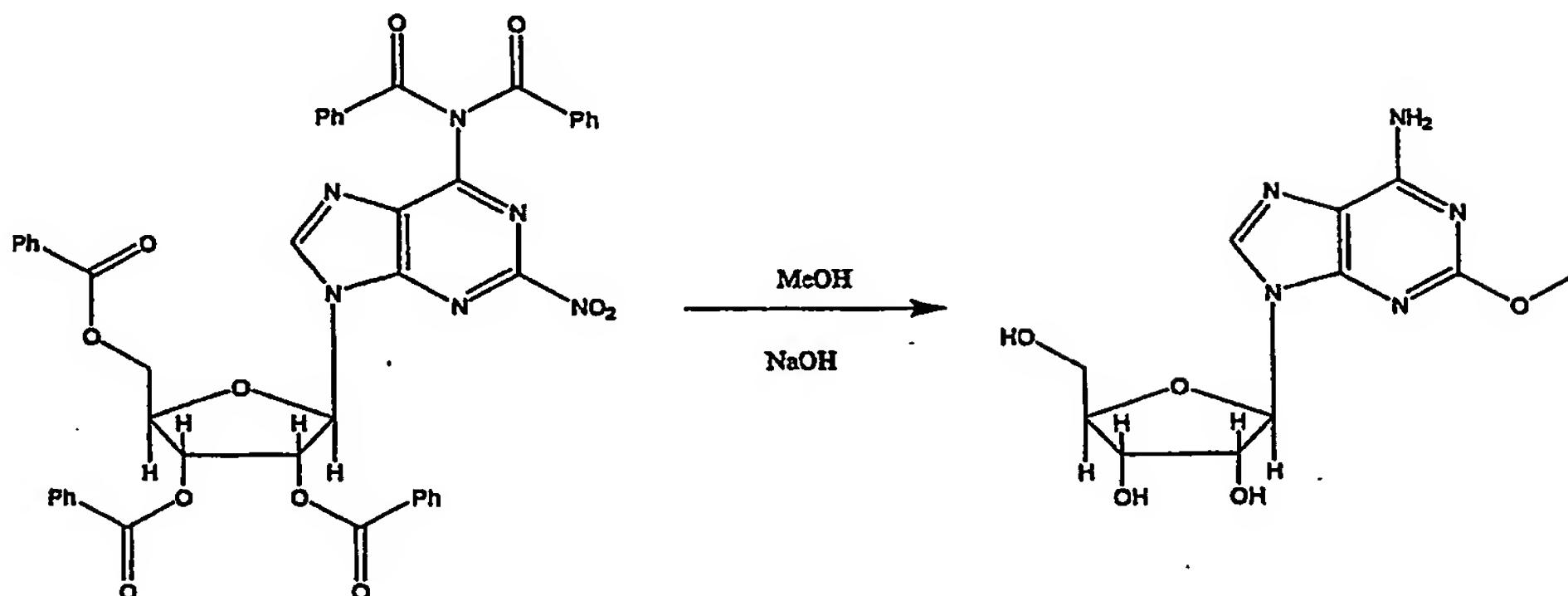
Preparation of 2-Nitro-Pentabenzoyl Adenosine using TBAN/TFAA as nitrating reagent:



To a solution of tetrabutylammonium nitrate (1.16 g, 3.81 mmol) in DCM (20 cm³) charge trifluoroacetic anhydride (0.80 g, 0.538 cm³, 3.81 mmol). Stir at 0 °C for 0.5h, then add a solution of pentabenzoyl adenosine (2.00 g, 2.54 mmol) in DCM (20 cm³) at 0 °C, cover reaction vessel in silver foil. Allow to warm to room temperature over 14h, reaction mixture poured onto ice/water, separate aqueous layer and extract with DCM (40 cm³), organic layers combined, solvent removed *in vacuo* [Temperature of rotary evaporator water bath is kept at 30 °C or below]. Residue dissolved in EtOAc (150 cm³), washed with water (5 × 75 cm³), brine (50 cm³), dried (MgSO₄). Solvent removed *in vacuo*, residue purified by recrystallisation from DCM/EtOH (twice) to give the desired product (1.604 g, 75.9 %) as a pale yellow solid. ¹H NMR (400MHz, CDCl₃): 4.79 (1H, dd, J = 11.5, 4.2 Hz), 4.92 (2H, m), 6.08 (1H, t, J = 5.6 Hz), 6.16 (1H, dd, J = 5.8, 4.4 Hz), 6.57 (1H, d, J = 5.4 Hz), 7.39 (10H, m), 7.55 (5H, m), 7.85 (4H, m), 7.92 (2H, m), 8.04 (4H, m) and 8.44 (1H, s). LCMS: 833 (M + H) and 855 (M + Na).

Example 4

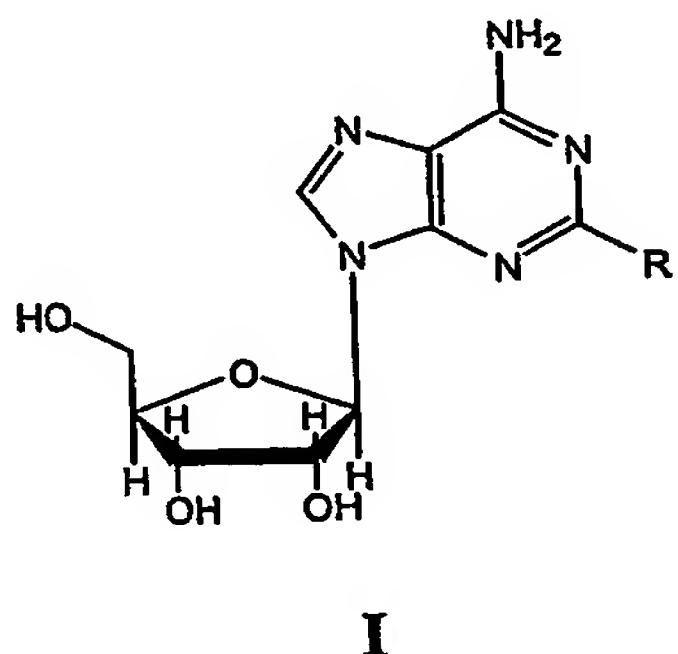
Preparation of 2-Methoxy Adenosine (Spongosine):



To a suspension of 2-nitro-pentabenzoyl adenosine (0.52 g, 0.62 mmol) in MeOH (10 cm³) charge a solution of NaOH (0.15 g, 3.70 mmol) in MeOH (10 cm³). Stir at room temperature for 16h, a red solution is obtained. Solvent removed *in vacuo*, residue dissolved in water and neutralised with 0.2M HCl (dropwise so as to prevent over acidification and resulting depurination). Solvent removed *in vacuo*, residue dissolved in MeOH: Water (1:1) (approx. 40 cm³) [requires heating], reaction mixture placed in freezer overnight (- 20 °C). Desired product precipitates out of reaction mixture, filtration gives the title compound (0.100 g, 54%) as a pale yellow solid. LCMS: 298 (M + H), small impurity 329 (M + H). Further purification can be carried out using reverse phase chromatography. ¹H NMR (400MHz, CDCl₃): 3.52 (1H, m), 3.60 (1H, m), 3.78 (3H, s), 3.89 (1H, dd, J = 7.2, 3.9 Hz), 4.12 (1H, m), 4.56 (1H, dd, J = 11.3, 6.1 Hz), 5.10 (1H, m), 5.11 (1H, d, J = 4.7 Hz), 5.35 (1H, d, J = 6.2 Hz), 5.75 (1H, d, J = 6.2 Hz), 7.27 (2H, br. s) and 8.11 (1H, s).

Claims

1. A method of synthesising a 2-substituted adenosine of formula I, which comprises converting 2-nitro-pentabenzoyl adenosine to the 2-substituted adenosine:



wherein R = C₁₋₆ alkoxy (straight or branched), a phenoxy group (unsubstituted, or mono-, or di-substituted by halo, amino, CF₃-, cyano, nitro, C₁₋₆ alkyl, or C₁₋₆ alkoxy), or a benzoyl group (unsubstituted, or mono-, or di-substituted by halo, amino, CF₃-, cyano, nitro, C₁₋₆ alkyl, or C₁₋₆ alkoxy).

2. A method according to claim 1, wherein R = methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, phenoxy, or benzoyl.

3. A method according to claim 1 or 2, wherein 2-nitro-pentabenzoyl adenosine is converted to the 2-substituted adenosine by deprotection, and reaction with C₁₋₆ alkoxide anion, or a phenoxide anion.

4. A method according to claim 3, wherein the anion is methoxide anion produced from MeOH/NaOMe, MeOH/n-BuLi, MeOH/NaOH, or MeOH/NaH.

5. A method according to any preceding claim, which further comprises converting pentabenzoyl adenosine to 2-nitro-pentabenzoyl adenosine.

6. A method of synthesising 2-nitro-pentabenzoyl adenosine which comprises converting pentabenzoyl adenosine to 2-nitro-pentabenzoyl adenosine.

7. A method according to claim 5 or 6, wherein pentabenzoyl adenosine is converted to 2-nitro-pentabenzoyl adenosine by nitrating pentabenzoyl adenosine using tetrabutylammonium nitrate (TBAN), or tetramethylammonium nitrate (TMAN) as nitrating reagent.
8. A method according to claim 7, which further comprises reducing the amount of TBAN or TMAN contaminating the 2-nitro-pentabenzoyl adenosine after the nitration reaction.
9. A method according to claim 8, wherein the amount of TBAN or TMAN is reduced by washing the 2-nitro-pentabenzoyl adenosine with water.
10. A method according to claim 9, which further comprises recrystallising the 2-nitro-pentabenzoyl adenosine after washing with water.
11. A method according to any of claims 5 to 10, which further comprises converting adenosine to pentabenzoyl adenosine.
12. A method of synthesising pentabenzoyl adenosine or 2-nitro-pentabenzoyl adenosine which comprises converting adenosine to pentabenzoyl adenosine.
13. A method according claim 11 or 12, wherein adenosine is benzoylated using benzoyl chloride.
14. 2-nitro pentabenzoyl adenosine
15. Use of 2-nitro pentabenzoyl adenosine in the synthesis of a 2-substituted adenosine of formula I.
16. Use of pentabenzoyl adenosine in the synthesis of 2-nitro-pentabenzoyl adenosine, or a 2-substituted adenosine of formula I.
17. Use of a benzoylating reagent in the synthesis of a 2-substituted adenosine of formula I.

18. A method of reducing the amount of TBAN or TMAN contaminating 2-nitro-pentabenzoyl adenosine formed by nitration of pentabenzoyl adenosine with TBAN or TMAN, which comprises washing the 2-nitro-pentabenzoyl adenosine with water.

19. A method according to claim 18 which further comprises recrystallising the 2-nitro-pentabenzoyl adenosine after washing with water.